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PATENT APPLICATION TRANSMITTAL LETTER

Docket Number: RPE-27

TO THE ASSISTANT COMMISSIONER FOR PATENTS:

Transmitted herewith for filing is the following patent application:

TITLE: METHOD OF IDENTIFYING
CHANGES IN BIOPOLYMERSPRIORITY CLAIMED TO: DE 98 06 303.2-52 filed 16 February 1998
DE 198 50 661.9 filed 03 November 1998

INVENTORS: Ilse CHUDOBA; Thomas LOERCH; and Andreas PLESCH SMALL ENTITY STATUS

Enclosed are:

- 17 Sheets of Specification
- 4 Sheets of drawings
- X Combined Declaration/Power of Attorney document (unsigned)
- Certified copy of priority documents
- X Two Verified Statements to establish small entity status under 37 CFR 1.9 and 1.27. (unsigned)
- Assignment
- Information Disclosure Statement; cited art; translation of pertinent portion of art
- IDS fee, if applicable

PATENT FILING FEES

BASIC FEE			\$760
CLAIMS IN EXCESS OF TWENTY (\$18 each)	<u>30</u> total claims	<u>10</u> extra claims	\$180
INDEPENDENT CLAIMS IN EXCESS OF THREE (\$78 each)	<u>2</u> independent claims	<u>0</u> extra claims	0
MULTIPLE DEPENDENT CLAIM (\$260 flat fee)	<u>0</u> multiple dependent claims		0
		TOTAL	\$940
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DateOscar A. Towler, III
Registration number 33,803Jc135 U.S. PTO
09/250466
02/16/99

Applicants: Chudoba et al.
Application: _____
Filed: Herewith
Title: METHOD OF IDENTIFYING CHANGES IN BIOPOLYMERS

Attorney Docket No.: RPE-27

VERIFIED STATEMENT CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) and 1.27(b)) - SMALL BUSINESS CONCERN

I hereby declare that I am

- ☐ the owner of the small business concern identified below:
☐ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN: MetaSystems, Hard & Software GmbH

ADDRESS OF CONCERN: Robert-Bosch-Straße 6, 68804 Altlussheim, Germany

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.12, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees to the United States Patent and Trademark Office, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention described in:

- ☒ the specification filed herewith, with title as listed above.
☐ the application identified above.
☐ the patent identified above.

If the rights held by the above identified small business concern are not exclusive, then each individual, concern or organization having rights in the invention must file separate verified statements averring to their status as small entities (37 CFR 1.27), and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d), or a nonprofit organization under 37 CFR 1.9(e). Each such person, concern or organization is:

- ☐ Listed below (full name and address).
☒ No such person, concern or organization exists.

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: _____

TITLE OF PERSON SIGNING IF OTHER THAN OWNER: _____

ADDRESS OF PERSON SIGNING: _____

SIGNATURE: _____ DATE: _____

Applicants: Chudoba et al.
Application: _____
Filed: Herewith
Title: METHOD OF IDENTIFYING CHANGES IN BIOPOLYMERS

Attorney Docket No.: RPE-27

VERIFIED STATEMENT CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) and 1.27(b)) - INDEPENDENT INVENTOR

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, to the Patent and Trademark Office described in:

☒ the specification filed herewith, title listed above

☐ the application identified above

☐ the patent identified above

I have not assigned, granted, conveyed, or licensed and am under no obligation under contract or law to assign, grant, convey, or license any rights in the invention to any person who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is

☒ Listed below (full name and address): *

MetaSystems
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68804 Altlussheim, Germany

☐ No such person, concern or organization exists.

*Note: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to saying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.289b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

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TITLE: METHOD OF IDENTIFYING CHANGES IN BIOPOLYMERS

FIELD OF THE INVENTION

The present invention relates to a method of identifying changes in biopolymers, especially in chromosomal DNA, using two or more different sets of labelled detector molecules, as well as to a diagnostic kit for detecting these changes.

BACKGROUND OF THE INVENTION

The representation of human chromosomes has been carried out so far with banding techniques which permit a specific recognition of the chromosomes using light and dark bands (*e.g.* G-banding, O-banding, R-banding). These banding techniques are based on methods developed by Caspersson *et al.*, (Exp. Cell Res. 80, 1970, 315-319), Sumner *et al.* (Nature 232, 1971, 31), Seabright *et al.* (Lancet 2, 1971, 971-972) and Dutrillaux *et al.* (C R Acad. Sci., Paris, 272, 1971, 3636-3640). However, the identity of individual chromosomal bands can not be defined in every instance with these methods since all bands of all chromosomes appear only either light or dark. This turns out to be a significant disadvantage since chromosomes can be very different morphologically from cell to cell and from tissue to tissue and can possibly comprise translocations (*e.g.*, in the case of tumors) the recognition of which can be of particular significance for the person to be examined. This applies, *e.g.*, to the decision whether or not to have children in the case of a parent having balanced translocations ("crossing-overs" or "exchanges of parts of chromosomes"), to the recognition of the cause of abnormalities in children with and without mental retardation, and to the diagnosis of leukemias and other tumors

which frequently exhibit specific chromosomal changes with diagnostic and therapeutic significance.

Fluorescence in-situ hybridization (FISH) was described for the first time for routine use in practicable form by Pinkel *et al.* (Proc. Natl. Acad. Sci. USA 83, 1986, 2934-2938) as a suggestion for solving this problem. Today, all human chromosomes of a metaphase can be represented in different colors with this method by using chromosome-specific DNA libraries (chromosome painting, 24-color FISH, Schröck *et al.*, Science 273, 1996, 496-497; Speicher *et al.*, Nature Genet. 12, 1996, 368-376). Through the use of vectors, *e.g.*, cosmids, pacs or YACs, which can contain different amount of human DNA, specific chromosomal regions can be re-checked with respect to their integrity via multicolor techniques by means of FISH. Even parts of genes and repetitive DNA elements can be identified in this way regarding their chromosomal localization and their presence or absence. However, a multicolor representation of chromosomal sections at the band level has not been possible so far.

Thus, the problem underlying the current invention is to provide a novel and improved method for the identification of, in particular, changes in chromosomal DNA which method enables a multicolor representation at the band level.

This problem is solved by the embodiments of the present invention characterized in the claims.

DETAILED DESCRIPTION OF THE INVENTION

In particular, a method of identifying changes in biopolymers as target molecules using two or more different sets of labelled detector molecules is provided in which at least two sets

are specific for a certain region in the target molecules and the labels of the particular detector molecules of these sets specific for a certain region in the target molecules are different, the method comprising the steps of

(a) carrying out bonding reactions between the detector molecules of the different sets and the target molecules, wherein the particular labelled detector molecules of at least two sets bond in such a manner to a certain region of the target molecules that the different labels of the detector molecules overlap, and

(b) qualitatively and quantitatively evaluating the bondings obtained in this manner via the different labels of the detector molecules.

The term "biopolymers as target molecules" means DNA (preferably chromosomal DNA), RNA, or polypeptides. The target molecules may be appropriately arranged or immobilized prior to carrying out the method of invention, in particular prior to step (a), *e.g.*, by gel electrophoretic separation in a suitable matrix or fixing or arranging, *e.g.*, metaphase chromosomes or interphase nuclei on a suitable carrier.

The term "labelled detector molecules" means nucleic acids or antibodies having at least one label. The antibodies may be present polyclonally or monoclonally. The terms "nucleic acid" and "nucleic-acid sequence" and "nucleic-acid probes" mean native, semisynthetic, or modified nucleic-acid molecules of deoxyribonucleotides and/or ribonucleotides and/or modified nucleotides such as amino nucleotides or (α -S)-triphosphate nucleotides. In a preferred

embodiment of the current invention the nucleic acids stem from chromosomal DNA from, *e.g.*, mammals such as *homo sapiens sapiens*. The chromosomal DNA as detector molecules is present in vectors, *e.g.*, cosmids or YACs, or stems from chromosomal or chromosome region-specific DNA libraries which can be obtained, *e.g.*, via microdissection methods or laser-activated flow-cytometric sorting of specific chromosomes and, if required, subsequent amplification by, *e.g.*, DOP-PCR.

The term "labels" means suitable directly or indirectly detectable atoms or molecules which are introduced into the detector molecules or connected to them. Suitable labels are, *e.g.*, those comprising fluorescent dyes coupled to nucleotides and/or those comprising, *e.g.*, biotin and/or digoxigenin and/or nucleotides labelled with radioactive isotopes. In a preferred embodiment the labelling compound is a fluorescent dye having a difference, sufficient for the selection of small amounts of substance, in the fluorescence behavior of the emission spectra such as, *e.g.*, cumarins and rodamins, and/or in the fluorescence lifespan such as, *e.g.*, fluorescent isothiocyanates and europium-chelate-labelled and/or porphyrin-labelled avidines.

The term "bonding reaction" means a hybridization, preferably an in situ hybridization, or an antigen/antibody reaction dependent on the selection of the detector molecules and/or of the target molecules. The term "in situ hybridization" means the apposition of a synthetically produced DNA and/or RNA molecule provided with biological, physical or chemical labels for detection as detector molecule to native DNA and/or RNA sequences occurring in nature, wherein the apposition is achieved by denaturing and renaturing the appropriate nucleic acids. Of course, these DNA and/or RNA probes contain at least one sequence section capable of hybridizing with a DNA and/or RNA sequence of the target molecule, such as a chromosome.

This sequence section comprises a specific, individually present sequence region of the detector molecule which region is preferably 100 to 1,000 base pairs long and which apposes itself to a complementary region of the target molecule through the formation of hydrogen bridges at a suitable temperature, preferably at 50 °C or less, and at a suitable saline concentration comprising preferably 50-300 mmol/l monovalent ions and 0-10 mmol/l bivalent ions. The bonding reaction of the particular sets of labelled detector molecules may be carried out simultaneously or successively.

The expression "set of detector molecules" means detector molecules which are specific for a certain region of the target molecules. This set of detector molecules may be, *e.g.*, chromosomal DNA present in vectors or may be a chromosome-specific DNA library. The labels of the detector molecules in the set may be the same or different, *e.g.*, three different labels.

The expression "at least two or more different sets of labelled detector molecules" means the presence of at least one pair of different sets, wherein the sets of this pair bond in a certain area or region of the target molecules in such a manner that at least the different labels of the particular detector molecules, preferably the bonding sites of the particular detector molecules of these different sets, overlap. This property, according to the invention, of a pair of different sets means that the particular detector molecules in the different sets of a pair which are produced or obtained in an overlapping manner from this certain region of the target molecules can be used as a standard or for comparative examination with appropriately processed specimens from patients. In an embodiment according to the invention the detector molecules of a set are preferably designed in such a manner that after the hybridization the detector

molecules are bound in a continuously changed concentration, preferably in the manner of a Gauss distribution, in the longitudinal direction to the target molecules, *e.g.*, chromosomes.

The qualitative and quantitative evaluation of the bondings obtained in step (a) via the different labels of the detector molecules, which evaluation is characterized in step (b) of the method according to the invention, may be accomplished by employing a scanning device or a device for directed scanning, *e.g.*, along or in the longitudinal direction of the chromosome to be investigated. Such a scanning device is, *e.g.*, a fluorescence microscope. Image-generating signals can be taken by the scanning device via an image processing unit, *e.g.*, a CCD camera, via the physical and/or chemical and/or biological labels of the detector molecules which have been apposed to the desired target molecules. This image processing unit processes the individual signals of the different labels in a suitable manner supported by a computer. The intensities and/or the intensity relationships of the different labels in the regions of overlapping and non-overlapping labels of the particular detector molecules can be recorded and evaluated qualitatively and quantitatively, preferably in the longitudinal direction of the target molecules, particularly of fixed metaphase chromosomes, with this image processing unit coupled to the scanning device.

Further subject matter of the current invention comprises a diagnostic kit for the identification of changes in biopolymers, as defined herein, as target molecules, the kit containing at least two different sets of labelled detector molecules in accordance with definitions set forth above.

In particular, the kit according to the invention can be used for the identification or exclusion of chromosomal aberrations in human genetics such as balanced chromosome

rearrangements which are, as is known, of great significance for (a) the decision whether or not to have children, in the case of carriers of such a change; (b) balanced and unbalanced chromosome changes as the cause of malformations and/or mental retardation; and (c) in the tumor diagnosis of solid tumors as well as of hematological neoplasias (AML, ALL, MDS, and others), on the one hand for the detection of known alterations relevant to prognosis and on the other hand for the determination of further, previously unknown alterations.

Further subject matter of the current invention relates to an automatic imaging correction by addition of a localized DNA probe.

A monochrome CCD camera in combination with specific fluorescence filters is used when recording chromosome region-specific specimens labelled with different fluorochromes like the specimens used for the methods of multicolor banding. The signals of the individual fluorochromes are recorded successively as individual images and subsequently combined to a color image. A shift of the position of the individual images relative to each other on account of optical influences of the filters (different wedge errors, parallel shift due to slight tilting in the path of the rays) can not be excluded thereby. An interactive or automatic correction, *e.g.*, by a correlation of the individual images, is not possible with the required precision because the at most partially overlapping probes do not have any common structures which can be used for a subsequent superpositioning. Every slight shift results in the evaluation of the intensity ratios in artifacts in the banding pattern.

An automatic correction is made possible by adding a localized DNA probe which is simultaneously labelled with all fluorochromes used in the method according to the invention. A structure which is identical in all individual images is therefore available for the automatic

correction of position. The correction of position may take place, *e.g.*, via a determination of the center of intensity of the probe in each individual image and by a subsequent relative shifting of the individual images in such a manner that the centers of the individual images come to be located at the same position.

5 Additionally, distortions of the images relative to each other can be determined and corrected by the use of two different multi-labelled probes.

The use of even more probes basically makes possible the correction of more complex transformations of position than translation and rotation such as, *e.g.*, changes of scale.

Furthermore, it can be advantageous in a further preferred embodiment of the current invention to add calibrating probes (DNA probes or fluorescent particles) of known intensity which can serve for standardizing the intensities of the fluorescent signals to be evaluated.

Furthermore, it can be advantageous in a further preferred embodiment of the current invention to add DNA probes whose exact localization within the genome is known and which can be used for establishing the relation between color bands and the ISCN bands.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a photographic representation for the qualitative and quantitative evaluation of the localization of region-specific colorations in chromosome 5. In the upper part of this figure the distribution of the labelled detector molecules in the longitudinal direction of the chromosome as well as intensities of the different labels of the detector molecules are shown graphically.

Figure 2 is a tabular presentation of the label pattern of the region-specific chromosome section of chromosome 5 shown in figure 1. Cy5, TR (Texas red), Cy5.5, SO (spectrum

orange), SG (spectrum green) are the different fluorescent dyes which were used to label the individual region-specific DNA libraries. The association is characterized by a solid square: (■). The labellings resulting from the overlapping of the DNA libraries in the corresponding regions are rendered recognizable by an empty square (□).

5 Figure 3 shows the respective homologous normal chromosomes 5 from two different metaphase plates with multicolored banding. The presentation makes it clear that the banding pattern is identical on the homologous chromosomes and can even be reproduced from metaphase plate to metaphase plate.

Figure 4 shows a photographic representation of a multicolored FISH of a metaphase plate with complex chromosomal aberrations.

Figure 5 shows chromosomes 5 in a case of acute myeloid leukemia. The normal chromosome 5 is shown on the right side and the chromosome on the left side displays an interstitial deletion in the long arm.

EXAMPLE

The following example explains the invention.

A total of seven overlapping chromosome microdissection region-specific libraries were produced for the multicolor band pattern of chromosome 5 (Meltzer et al., Nature Genet. 1, 1992, 24-26). The *p* arm of chromosome 5 was subdivided for this into two regions, the *q* arm into four regions. Eight to ten fragments per chromosome region were isolated with a finely drawn-out glass needle from the microscope slide under microscopic view (Senger et al., Hum, Genet. 84, 1990, 507-511). The thus obtained DNA was amplified via a DOP-PCR (degenerate

oligonucleotide polymerase chain reaction, Telenius et al., Genomics 13, 1992, 718-725; Zhang et al., Blood 81, 1993, 3365-3371). In a subsequent reaction these chromosome region-specific DNA libraries were partially labelled directly with fluorochromes coupled to nucleotides (*e.g.*, Spectrum Orange-dUTP, Spectrum Green-dUTP, both Vysis and Texas Red-dUTP, Molecular Probe). In another part, DNA libraries were labelled with nucleotides coupled to haptens (*e.g.*, biotin-dUTP and digoxigenin-dUTP, Boehringer, Mannheim). After the hybridization has taken place haptens can be detected with suitable detection reagents (*e.g.*, avidine-Cy5, Amersham, and anti-digoxigenin IgG, Boehringer, Mannheim, which is coupled to Cy5.5, Mab labeling kit, Amersham).

The hybridization, washing steps, and detection are carried out according to standard protocols (Senger et al., Cytogenet. Cell Genet. 54, 1993, 49-53).

The analysis is carried out, *e.g.*, with a fluorescence microscope equipped with suitable filter sets. Separate images are taken for each color channel, which images can be subsequently processed further with a computer.

A characteristic feature of the partial "painting" probes obtained by microdissection is a continuously weakening fluorescent signal in the border regions. A simultaneous overlapping of the probes and, therefore, of the fluorescent signals of two adjacent partial "painting" probes brings about a constantly changing ratio of the fluorescence intensities along chromosome 5. If a chromosome stained in this manner is subdivided into several (20-25) small sections, a false color stain can be assigned to each of these sections via a suitable computer program on the basis of the relative fluorescence intensities of all fluorochromes used. This assignment gives rise to a colored band pattern along a chromosome, in this case chromosome 5. The same combination

of fluorescence relationships and false colors can be used for all further hybridizations with the same specimen set.

Since the hybridization behaves in a sufficiently constant manner the band pattern is also correspondingly reproducible (figure 3). A loss of the resolving power in the case of shorter chromosomes, as is known from previous customary banding methods (*e.g.*, GTG banding) is not observed in this case. A reproducible pattern of at least 25 bands is achieved for chromosome 5. This corresponds to a band level of approximately 550 bands per haploid chromosome set.

It is possible with the aid of this method to identify changes in chromosomes independently of their condensation state. This is particularly significant in tumor cytogenetics, too. Tumor chromosomes often display a low resolution of the band pattern, which makes the recognition of chromosomal changes significantly difficult. It is therefore to be assumed that previously unknown cytogenetic changes are present in tumors, which possibly represent an important prognosis factor and could therefore be of significance, *e.g.*, for a risk-adapted therapy. According to the invention at least 25 bands can be achieved even on tumor chromosomes after hybridization with the specimen set for chromosome 5 described in detail above (figure 5).

What is claimed is:

1. A method of identifying changes in biopolymers, the method comprising the steps:

(a) providing different sets of labelled detector molecules in which:

at least two sets of said labelled detector molecules are specifically

5 bondable to a certain region in said biopolymers; and

the labels of said labelled detector molecules of one of said at least two sets differ from the labels of said labelled detector molecules of another of said at least two sets;

(b) exposing said labelled detector molecules to said biopolymers under conditions permitting bonding reactions to occur to form bondings between said labelled detector molecules and said biopolymers; and

(c) evaluating said bondings via said different labels whereby changes in said biopolymers may be identified.

2. The method according to claim 1, wherein said biopolymers are immobilized at least before step (b).

3. The method according to claim 2, wherein said biopolymers are fixedly arranged on a carrier or in a matrix.

4. The method according to claim 1, wherein said bonding reactions between each of said labelled detector molecules and said biopolymer are carried out simultaneously or successively.

5. The method according to claim 1, wherein said bonding reaction in step (b) is a nucleic acid hybridization or an antigen/antibody reaction.

6. The method according to claim 5, wherein said nucleic acid hybridization is an *in situ* hybridization.

7. The method according to claim 1, wherein said biopolymers are nucleic acids or polypeptides.

8. The method according to claim 7, wherein said nucleic acids are DNA or RNA.

9. The method according to claim 7, wherein the nucleic acids are chromosomal DNA.

10. The method according to claims 1, wherein the labelled detector molecules are nucleic acids or antibodies.

11. The method according to claim 10, wherein said different nucleic acids stem from different chromosome region-specific DNA libraries.

12. The method according to claim 10, wherein each of said sets of labelled detector molecules contains one or more labels different from at least one label contained in another of said sets.

13. The method according to claim 12, wherein the label comprises a fluorescent dye.

14. The method according to claim 1, wherein said evaluating step further comprises the steps:

scanning said biopolymers with a scanning device in the longitudinal direction of said biopolymers; and

recording the intensities or intensity ratios of said labels of said labelled detector molecules.

15. A diagnostic kit for identifying changes in biopolymers comprising:
a first set of detector molecules labelled with a first label;
a second set of detector molecules labelled with a second label, said second label
differing from said first label;

5 said first and second sets of detector molecules capable of forming bondings with
biopolymers whereby evaluation of said bondings permits identification of changes in said
biopolymers.

16. The diagnostic kit according to claim 15, wherein said sets of detector molecules
are capable of forming bondings with material selected from the group consisting of human
chromosomal material and tumor material.

17. The method according to claim 1, wherein said step of providing different sets
of labelled detector molecules further comprises providing at least one set of a localized
calibrating probe, said probe comprising calibrating labels.

18. The method according to claim 17, wherein said calibrating labels comprise all
of said labels of said labelled detector molecules of said at least two sets.

19. The method according to claim 1, wherein said step of providing different sets
of labelled detector molecules further comprises providing a number of localized calibrating
probes, said number being one less than the total number of said labels in said labelled detector
molecules, each of said probes comprising two labels; and said evaluating step further
comprising correcting positional deviations of said bondings by pairwise comparison of said
calibrating probes.

20. The method according to claim 17, wherein positional transformations of said bondings are corrected by using a sufficient number of said probes.

21. The method according to claim 17, wherein determination of the relative shifts and positional correction of said bondings take place interactively during said step of evaluating.

22. The method according to claim 17, wherein determination of the relative shifts and positional correction of said bondings take place automatically.

23. The method according to claim 17, wherein said labels of said calibrating probes have known or reproducible constant intensity whereby the signal intensities of all of said labels can be standardized.

24. The method according to claim 23, wherein said calibrating probes are fluorescence-labelled DNA probes.

25. The method according to claim 23, wherein said calibrating probes are fluorescence-labelled particles.

26. The method according to claim 17, wherein said calibrating probes are simultaneously used for positional correction.

27. The diagnostic kit according to claim 15, further comprising calibrating probes.

28. The diagnostic kit according to claim 27, further comprising calibrating probes for intensity standardization.

29. The diagnostic kit according to claim 27, further comprising a sufficient number of said calibrating probes to enable a direct assignment of color bands to the ISCN nomenclature.

30. The diagnostic kit according to claim 27, wherein said probes are simultaneously used for positional correction or intensity standardization.

ABSTRACT:

The current invention relates to a method of identifying changes in biopolymers, especially in chromosomal DNA, using two or more different sets of labelled detector molecules, as well as to a diagnostic kit for detecting these changes.

Fig. 1

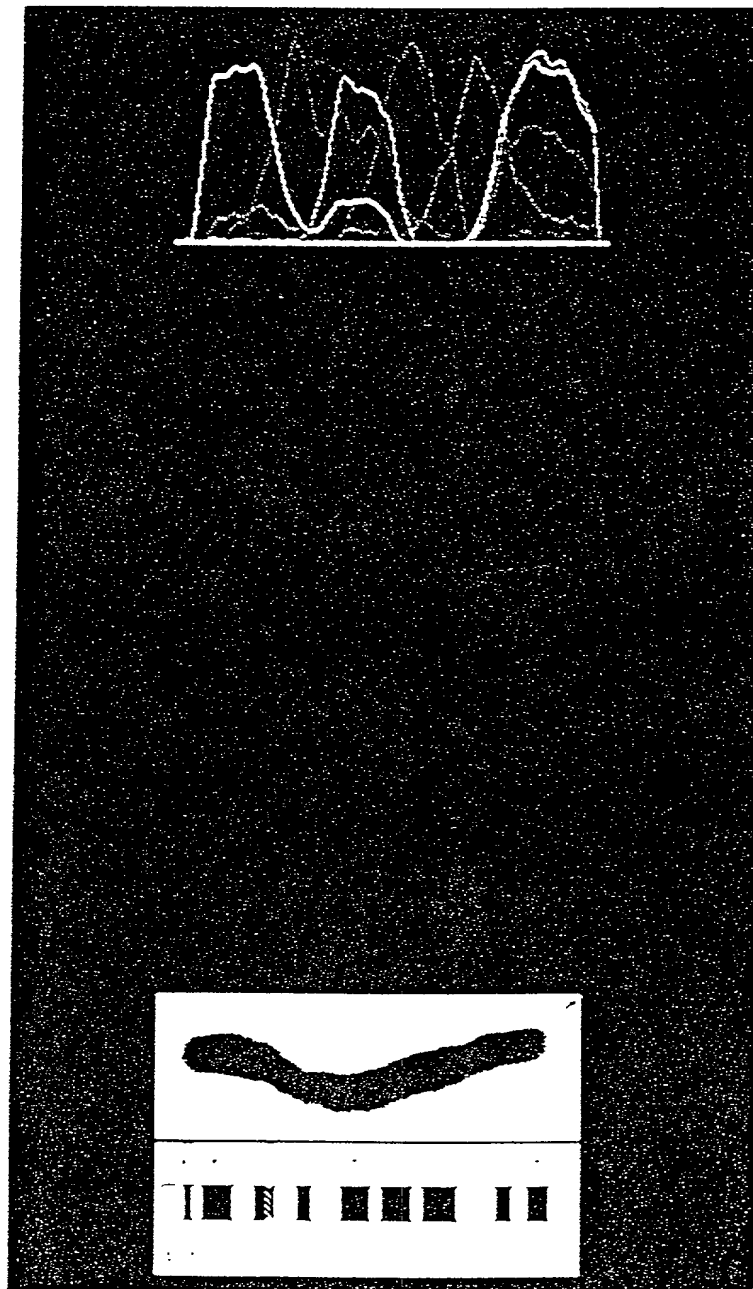


Fig. 2

region-specific DNA library	Cy 5	TR	Cy 5.5	SO	SG
1	■				
1/2	□	□			
2		■			
2/3		□	□		
3			■		
3/4			□	□	
4				■	
4/5				□	□
5					■
5/6	□		□		□
6	■		■		

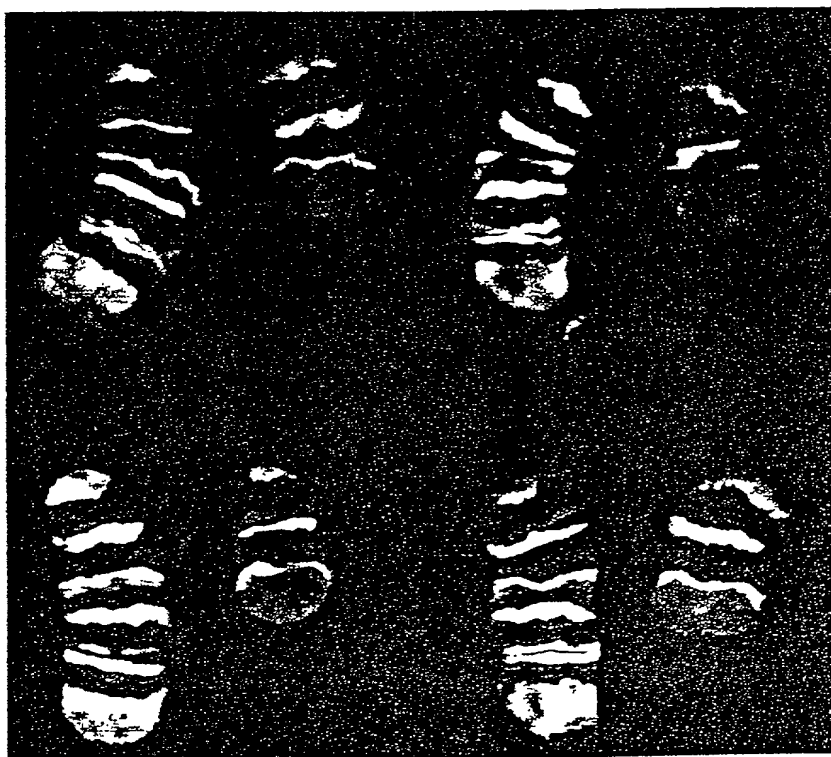


Fig. 4

Fig. 3



Fig. 5



COMBINED DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION

Attorney Docket
Number: **RPE-27**

DECLARATION: As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled
METHOD OF IDENTIFYING CHANGES IN BIOPOLYMERS

the specification of which:

☒ is attached hereto.

☐ was filed on _____ as U.S. Application Number _____ or on _____
as PCT Application Number _____ and amended on _____ (if applicable.)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119 (a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application which designated at least one country other than the United States listed below, and have also identified below any foreign application for patent or inventor's certificate or PCT international application having a filing date before that of the application on which priority is claimed:

			Priority Claimed	
198 06 303.2-52	Germany	16 February 1998	<input checked="" type="checkbox"/>	<input type="checkbox"/>
(Number)	(Country)	Filed (Day/Month/Year)	Yes	No
198 50 661.9	Germany	03 November 1998	<input checked="" type="checkbox"/>	<input type="checkbox"/>
(Number)	(Country)	Filed (Day/Month/Year)	Yes	No

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

Application Serial No.)	(Filing Date)	Application Serial No.)	(Filing Date)
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I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or §365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR §1.56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

Application Serial No.)	(Filing Date)	(Status - patented, pending, abandoned)
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Application Serial No.)	(Filing Date)	(Status - patented, pending, abandoned)
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POWER OF ATTORNEY: I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: John B. Hardaway, III, Reg. No. 26,554; Katja Dunbar Peralta, Reg. No. 40,768, Sandra S. Snapp, Reg. No. 41,444; Oscar A. Towler, III, Reg. No. 33,803; Jacquelyn D. Austin, Reg. No. 43,478; J. Herbert O'Toole, Reg. No. 31,404; and George E. Darby, Reg. No. P-44,053.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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